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CONCERNING NATURAL HEMOLYSINS IN RABBIT SERUM.*

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During the course of some work on the site of formation of immune hemolysins, the results of which will be published in a later communication, it was necessary to determine whether or not the serum of the animal about to be immunized contained any natural hemolytic amboceptors to the erythrocytes which were to be injected.

Rabbits, immunized with sheep's corpuscles, were used. Before starting the injections, the sera of the rabbits were tested for natural anti-sheep hemolysins and in 16 of the 18 used, or 88 per cent, their presence was demonstrated.

This result was unexpected, and accordingly we titrated an additional 32 normal rabbit sera to determine if such a large percentage were accidental. The result is shown in Table 1.

A fairly minute search of literature failed to give us any reference to observations along this line. Bordet[1] states that "it is a general rule, applicable to all sera, that the power to destroy red blood corpuscles disappears on heating to 55° C." Subsequently he says: "Guinea-pig serum and rabbit serum have both a very slight destructive effect on hen and human corpuscles. Rabbit serum, however, is much more active against guinea-pig corpuscles, but is almost entirely without effect on rat corpuscles . . . . . Normal rabbit serum contains a sensitizer for goat corpuscles." Ehrlich[2] observes that rabbit serum is often hemolytic to goat red blood corpuscles and that it contains a very variable amount of hemolysin for guinea-pig blood.

After observing the almost constant occurrence of natural anti-sheep hemolysin in rabbit serum, we decided it would be interesting to determine if it contained native hemolytic amboceptors for the

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[1] *Studies in Immunity* (Wiley & Sons).

[2] *Studies in Immunity* (Wiley & Sons).

erythrocytes of the following vertebrates; chicken, dog, goat, guinea-pig, hog, man, ox, and white rat.

Preparation of materials.—The rabbits were bled from an ear and the serum separated by centrifugalization from the clot after one hour's standing. The sera were kept in sterile glass ampoules. Before testing, the serum was inactivated by heating to 55° C., for 30 minutes, in order to destroy any anticomplementary substances, as well as native complement. This inactivated serum was then diluted with 9 parts of salt solution (0.85 per cent) and used in the following amounts 0.05 c.c., 0.2 c.c., 0.4 c.c., 0.8 c.c., 1 c.c., and 2 c.c., corresponding respectively to 0.005, 0.02, 0.04, 0.08, 0.1, and 0.2 c.c. of the undiluted serum. Complement was furnished by fresh guinea-pig serum, using 1 c.c. of a 5 per cent dilution in normal saline. The corpuscles were used in dose of 1 c.c. of a 2.5 per cent suspension in all the tests, with the exception of the chicken cells, which was a 2 per cent, and the white rat, which was a 1.7 per cent suspension.

Technic.—In tube 1, was placed 0.05 c.c. of the 1:10 inactivated rabbit serum; in tube 2, 0.2 c.c.; in tube 3, 0.4 c.c.; in tube 4, 0.8 c.c.; in tube 5, 1 c.c.; and in tube 6, 2 c.c. Then were added 1 c.c. of the 1:20 guinea-pig complement and 1 c.c. of the corpuscle suspension and enough normal salt solution to make the total volume 4 c.c. After shaking, the tubes were placed in the incubator at 37° C., for 2 hours, removed, and allowed to stand in the refrigerator over night and the results read in the morning.

Controls.—In every instance the guinea-pig serum used as complement was tested for natural hemolysin for the erythrocytes of the animal being tested. Guinea-pig serum frequently contains an appreciable amount of natural anti-sheep amboceptor but when used in dosage of 1 c.c. of 1:20 dilution, the amount of natural amboceptor for the various red cells of the other animals used was not sufficient to require removal before the serum could be used for complement. Each serum was tested in maximum dosage with the corpuscles to make sure that all native complement was rendered inactive; with several sets of reactions, specific immune hemolysins were used with the sera to make certain that none of the sera were anticomplementary. The corpuscle suspensions were always prepared of fresh blood and controlled as to undue fragility, by suspending a dose in salt solution.

Natural anti-sheep hemolysin.—The sera obtained from 50 normal rabbits were tested with the results as shown in Table 1.

TABLE 1.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	2	10	24
0.02	2	8	20	"
0.04	4	10	40	"
0.08	6	22	56	"
0.10	14	40	68	"
0.20	18	56	76	"

It is evident that inactivated rabbit serum contains considerable natural anti-sheep hemolysin; 38 of the 50 sera examined exhibiting it. In 2 per cent of them 0.02 c.c. of serum was sufficient to cause complete hemolysis and in 12 per cent such a small amount as 0.005 c.c. of serum caused from 25 to 75 per cent of hemolysis. It is generally recognized that some rabbits yield a higher output of immune hemolysis than others; but the difference may be largely due to this observation concerning the high content of natural hemolysin in 10 to 12 per cent of rabbits.

Natural anti-human hemolysin.—Fifty rabbit sera were tested against human erythrocytes with results as tabulated below.

TABLE 2.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	80
0.02	0	0	8	"
0.04	0	0	12	"
0.08	0	0	12	"
0.10	0	2	14	"
0.20	0	4	20	"

Here we note that 40 of the 50 sera showed no hemolysin when used in amounts up to 0.2 c.c. and of the 10 giving hemolysis none was able to cause complete destruction, one gave 75 per cent hemolysis in an amount of 0.1 c.c., and four showed slight (25 per cent) hemolysis in doses of 0.02 c.c.

Anti-goat hemolysin.—Twenty-five rabbits were examined for natural anti-goat hemolysin.

TABLE 3.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	4	12
0.02	0	4	16	"
0.04	0	4	44	"
0.08	0	8	72	"
0.10	0	28	76	"
0.20	4	52	88	"

Only three of these sera contained no hemolytic amboceptors. Of the remaining 22 which exhibited hemolysis, one showed complete hemolysis with 0.2 c.c. of serum and 13 gave 75 per cent hemolysis with the same amount. The smallest amount of serum causing any hemolysis was 0.005 c.c.

Anti-dog hemolysin.—Table 4 shows the results of examining 25 sera for anti-dog hemolysin.

TABLE 4.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	28
0.02	0	0	4	"
0.04	0	8	16	"
0.08	0	16	48	"
0.10	12	32	52	"
0.20	32	40	72	"

Here we note that the anti-dog hemolytic amboceptors contained in normal rabbit serum are more potent than any of the others studied, but their presence is not so constant nor will such small amounts of serum cause hemolysis, it requiring at least 0.02 c.c. to cause 25 per cent hemolysis. However, in 12 per cent of the cases showing hemolysis, 0.1 c.c. of serum causes complete destruction of the dog's red blood corpuscles.

Anti-hog hemolysin.—Twenty-five sera were tested for anti-hog hemolysin (Table 5).

Twenty of these failed to show any trace of amboceptor. Of the 5 sera causing hemolysis, none gave complete hemolysis with the

TABLE 5.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	80
0.02	0	0	0	“
0.04	0	0	4	“
0.08	0	0	8	“
0.10	0	0	20	“
0.20	0	4	20	“

largest amount of serum used and 0.04 c.c. was the smallest amount which could dissolve 25 per cent of the hog's erythrocytes.

Anti-ox hemolysin.—Table 6 gives the results of examining 25 sera for anti-ox hemolysin.

TABLE 6.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	80
0.02	0	0	4	“
0.04	0	0	8	“
0.08	0	0	8	“
0.10	0	0	16	“
0.20	0	4	20	“

The amount of natural anti-ox hemolytic amboceptor appears to be about the same as the anti-human and the anti-hog hemolysins already commented on.

Anti-chicken hemolysin.—Twenty-five sera were tested for anti-chicken hemolytic amboceptors.

TABLE 7.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	92
0.02	0	0	0	“
0.04	0	0	0	“
0.08	0	0	4	“
0.10	0	0	4	“
0.20	0	4	8	“

Only two sera exhibited any hemolysin. At least 0.08 c.c. of serum was necessary to give 25 per cent hemolysis and in the other case the largest amount of serum used, 0.2 c.c. caused 75 per cent hemolysis.

Anti-guinea-pig hemolysis.—Table 8 shows the examination of 25 rabbit sera for anti-guinea-pig hemolysin.

TABLE 8.

Amount of Serum c.c.	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
0.005	0	0	0	96
0.02	0	0	0	"
0.04	0	0	0	"
0.08	0	0	0	"
0.10	0	0	0	"
0.20	0	0	4	"

Twenty-four of the sera showed no trace of hemolysin and the only one giving hemolysis required the largest amount of serum used, 0.2 c.c., to cause a 25 per cent hemolysis.

Anti-rat hemolysin.—Twenty-five sera were tested for anti-white rat hemolysin with negative results in all cases. The white rat (*Mus norvegicus*, var albinus) is the only animal examined for which rabbit serum caused no destruction of its red blood cells.

The foregoing tables, with the accompanying remarks, give all the essential data, but it will be of interest to analyze the results more minutely and determine what were the smallest amounts of normal rabbit's serum causing 100 per cent, 75 per cent, and 25 per cent destruction of the red blood cells of the various animals investigated and what percentage they bear to the number of sera tested. These results are shown in the accompanying table.

TABLE 9.

SHOWING SMALLEST AMOUNTS OF RABBIT'S SERUM GIVING 100, 75, AND 25 PER CENT HEMOLYSIS.

Blood	100% Hemolysis	75% Hemolysis	25% Hemolysis
Chicken	....	0.2 c.c. (4%)	0.08 c.c. (4%)
Dog	0.1 c.c. (12%)	0.04 " (8%)	0.02 " (4%)
Goat	0.2 c.c. (4%)	0.02 " (4%)	0.005 " (4%)
Guinea-pig	....	....	0.2 " (4%)
Hog	....	0.2 " (4%)	0.04 " (4%)
Human	....	0.1 " (2%)	0.02 " (8%)
Ox	....	0.2 " (4%)	0.02 " (4%)
Rat—white	....	....	....
Sheep	0.02 c.c. (2%)	0.005 " (2%)	0.005 " (10%)

It will be noted that as small an amount as 0.005 c.c. of rabbit serum contains enough amboceptor to cause 25 per cent hemolysis in 10 per cent of the tests for anti-sheep hemolysin and 4 per cent of the anti-goat determinations. Also, that the smallest amount giving complete hemolysis was 0.02 c.c. in the case of sheep's corpuscles, 0.1 c.c. with dog's red blood cells, and 0.2 c.c. with the goat's erythrocytes.

In conclusion, we have arranged the various bloods under investigation in the order in which rabbit serum contains the most constant amount of natural hemolytic amboceptors for them.

TABLE 10.

SUMMARY OF NATURAL HEMOLYSINS IN NORMAL RABBIT SERUM.

Blood	Number of Sera Tested	100% Hemolysis Percentage	75% Hemolysis Percentage	25% Hemolysis Percentage	0 Hemolysis Percentage
Goat	25	4	52	88	12
Sheep	50	18	56	76	24
Dog	25	32	40	72	28
Human	50	0	4	20	80
Hog	25	0	4	20	80
Ox	25	0	4	20	80
Chicken	25	0	4	8	92
Guinea-pig	25	0	0	4	96
Rat—white	25	0	0	0	100